



Dryland soil carbon dynamics under alfalfa and durum-forage cropping sequences

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ABSTRACT

Forages grown in rotation with or without cereals to sustain dryland soil water content and crop production may influence C dynamics. We evaluated the effect of alfalfa (*Medicago sativa* L.) and durum (*Triticum turgidum* L.)-annual forage cropping sequences on above- (stems + leaves) and belowground (roots) biomass C inputs and dryland soil organic C (SOC), particulate total C (PTC), microbial biomass C (MBC), and potential C mineralization (PCM) at the 0–120 cm depth. Cropping sequences were continuous alfalfa (CA), durum-barley (*Hordeum vulgare* L.) hay (D-B), durum-foxtail millet (*Setaria italica* L.) hay (D-M), durum-Austrian winter pea (*Pisum sativum* L.)/barley mixture hay (D-P/B), and durum-fallow (D-F). The experiment was conducted in a Williams loam (fine-loamy, mixed, superactive, Typic Argiustoll) from 2002 to 2005 in eastern Montana, USA. Except in 2003, aboveground biomass yield and C content were lower in CA than in other treatments from 2002 to 2005. Similarly, belowground biomass yield and C content were lower in D-F than in other treatments from 2003 to 2005. In 2005, soil surface residue amount and C content were greater in D-F than in other treatments. The SOC at 0–15 cm was greater in CA than in D-B and D-M. The PTC at 0–15 cm was greater in CA than in other treatments, but varied with treatments at other depths. The PCM at 0–120 cm was greater in CA than in other treatments. The MBC at 30–120 cm was greater in CA and D-P/B than in D-B. The proportion of SOC in PTC, PCM, and MBC at 0–120 cm was greater in CA or D-P/B than in D-B and D-F. Although aboveground biomass C input was lower, greater belowground biomass C and a relatively undisturbed soil condition probably increased C storage at the surface layer and microbial biomass and activity at the surface and subsurface layers under alfalfa than under annual durum-forage sequences. Greater aboveground biomass C and fresh residue accumulation during durum phase, however, increased C storage in the surface residue under durum-fallow than under other cropping sequences. Perennial forages, such as alfalfa, can increase dryland soil C sequestration and biological soil quality by increasing microbial biomass and activity compared with annual cropping systems due to greater belowground biomass C input and continuous root growth.

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1. Introduction

In dryland cropping systems, crop-fallow has been the conventional farming system for the last several decades in the northern Great Plains, USA (Haas et al., 1974; Peterson et al., 1993). Immediately after fallowing, the alternate-year fallow system conserves soil water which is one of the main factors for crop production in dryland cropping systems where precipitation is limited. Other benefits of fallowing include weed control and nutrient mineralization that can influence succeeding crop yields (Aase and Pikul, 1995; Jones and Popham, 1997). Extending the fallow period by reducing cropping intensity, however, can result in reduced soil water-use efficiency, increased saline seeps development, and enhanced soil organic matter mineralization due to increased soil water and temperature during fallow (Haas

et al., 1974; Black and Bauer, 1988). The system has not only decreased crop yields and became uneconomical by the absence of crops during fallow (Aase and Schaefer, 1996; Dhuyvetter et al., 1996) but also reduced soil quality and productivity due to loss of soil organic matter from increased soil erosion (Haas et al., 1974; Aase and Pikul, 1995; Halvorson et al., 2002a).

One of the options to reduce the fallow period and increase water-use efficiency, crop yields, and net returns in dryland cropping systems is continuous cropping, such as cereal-annual forage sequences (Farhani et al., 1998). Inclusion of annual forages in rotation with cereals can maintain both cereal and forage yields because forages are harvested earlier for hay than cereals, which result in sustained soil water content and succeeding crop yields (Entz et al., 2002; Pikul and Aase, 2003). Continuous cropping can also increase the amount of crop residue returned to the soil and organic matter compared with crop-fallow (Aase and Pikul, 1995; Sainju et al., 2007, 2009). Rotating perennial legume forages with cereals can enrich soil N and control weeds and pests (Entz et al., 2002).

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Although grains are harvested and biomass (stems + leaves) is usually returned to the soil in the cereal production system, aboveground biomass is harvested for hay in the forage production system. Differences in the amount of above- and belowground biomass (root and rhizosphere deposition) C inputs returned to the soil between cereal and forage production systems can influence C storage and soil quality. Roots can contribute as much as 20–56% of the aboveground plant biomass, depending on crop species and stages of plant growth (Amos and Walters, 2006; Johnson et al., 2006). Roots can supply from 350 to 3800 kg C ha⁻¹ during a growing season (Qian and Doran, 1996; Johnson et al., 2006). Liang et al. (2002) found that roots contributed as much as 12% of soil organic C (SOC), 31% of water soluble C, and 52% of microbial biomass C (MBC) within a growing season. Roots may play a dominant role in soil C cycle (Gale et al., 2000; Puget and Drinkwater, 2001) and may have relatively greater influence on SOC than the aboveground plant biomass (Puget and Drinkwater, 2001). Balesdent and Balabane (1996) observed that corn (*Zea mays* L.) roots contributed 1.6 times more C to SOC than did by stover. Root-derived C is retained and forms more stable soil aggregates than shoot-derived C (Gale et al., 2000). Growing plants tend to maintain SOC level by continuously supplying C from roots compared with bare soil which tends to decrease it (Sanchez et al., 2002).

To increase C sequestration and biological soil quality, a better understanding of soil C cycling is needed. Some of the parameters of soil C cycling are SOC, particulate total C (PTC), MBC, and potential C mineralization (PCM). Since SOC has a large pool size and inherent spatial variability, it changes slowly with management practices (Franzluebbers et al., 1995). As a result, measurement of SOC alone does not adequately reflect changes in soil quality and nutrient status (Franzluebbers et al., 1995; Bezdicsek et al., 1996). Measurement of biologically active fractions of SOC, such as MBC and PCM, that change rapidly with time could better reflect changes in soil quality and productivity that alter nutrient dynamics due to immobilization–mineralization (Saffigna et al., 1989; Bremner and Van Kessel, 1992). These fractions can provide an assessment of soil organic matter changes induced by management practices, such as tillage and cropping systems (Campbell et al., 1989). Similarly, PTC has been considered as an intermediate fraction of SOC between active and slow fractions that changes due to management practices (Cambardella and Elliott, 1992). The PTC also provides substrates for microorganisms and influences soil aggregation (Six et al., 1999).

Little is known about the effect of perennial legume forages, such as alfalfa, and cereal–annual forage cropping sequences on soil C dynamics under dryland cropping systems in the northern Great Plains, USA. We hypothesized that alfalfa and durum–annual forage sequences would increase soil C storage and microbial biomass and activity compared with durum–fallow due to greater amount of crop residue C returned to the soil. Our objectives were to: (1) quantify the amount of above- and belowground (0–120 cm depth) biomass C returned to the soil in alfalfa and durum–annual forage sequences from 2002 to 2005 and (2) evaluate the effect of cropping sequence on dryland surface residue C and soil labile and nonlabile C fractions (SOC, PTC, MBC, and PCM) in the northern Great Plains, USA.

2. Materials and methods

2.1. Experimental site and treatments

The study was conducted from 2002 to 2005 in a dryland farm site, 11 km north of Culbertson, MT, USA (48°16' N, 104°30' W) at an altitude of 660 m. The climate is semiarid, with mean monthly air temperature ranging from –8 °C in January to 23 °C in July and

Table 1

Monthly and growing season (April–October) total precipitation (mm) from 2002 to 2005 at the experimental site.

Month	2002	2003	2004	2005	105-yr average
January	10.2	13.5	23.9	10.7	9.1
February	0.0	8.4	22.9	1.0	6.9
March	14.5	24.6	6.1	11.7	11.4
April	12.7	16.8	19.6	0.0	24.1
May	31.0	69.1	72.6	79.0	49.5
June	85.6	113.8	29.7	171.5	76.9
July	45.7	42.4	84.1	41.7	53.8
August	38.4	30.5	63.8	29.2	35.8
September	19.8	25.9	20.3	35.6	32.5
October	20.6	17.5	17.3	25.7	20.1
November	0.0	6.6	2.8	19.1	10.7
December	13.0	15.0	15.0	8.9	8.6
Total	291.3	409.4	378.0	401.1	340.1
April–October	253.8	316.0	307.4	382.7	292.7

August. The mean annual precipitation (105-yr average) is 340 mm, 80% of which occurs during the crop growing season (April–September) (Table 1). The soil is a Williams loam (fine-loamy, mixed, superactive, Typic Argiustoll) with 2–8% slope. The soil sampled in 2001 prior to the initiation of the experiment had 660 g kg⁻¹ sand, 180 g kg⁻¹ silt, 160 g kg⁻¹ clay, 1.50 Mg m⁻³ bulk density, 17.4 Mg ha⁻¹ SOC, and 6.1 pH at the 0–15 cm depth. Previous cropping history was spring wheat (*Triticum aestivum* L.)–fallow from 1984 to 1998, durum in 1999 and 2001, and lentil (*Lens culinaris* L.) in 2000.

Treatments consisted of four two-year cropping sequences containing durum followed by annual forages and a perennial legume forage alfalfa (CA). The two-year cropping sequences were durum–barley hay (D-B), durum–foxtail millet hay (D-M), durum–Austrian winter pea/barley mixture hay (D-P/B), and durum–fallow (D-F). The D-P/B contained durum sequenced by an interseeded mixture of Austrian winter pea and barley harvested for hay. The D-F was considered the conventional cropping system. Each phase of the cropping sequence was present in every year. Treatments (five treatments containing alfalfa and two phases of four durum–annual forage sequences) were arranged in randomized complete block with three replications. Individual plot size was 61 m by 21.3 m.

2.2. Crop management

Before planting in April of each year from 2002 to 2005, N fertilizer as urea (46% N) and monoammonium phosphate (11% N, 52% P) were applied to durum at 118 kg N ha⁻¹ and to annual forages at 78 kg N ha⁻¹. Soil NO₃–N content to a depth of 60 cm determined after crop harvest in October of the previous year was deducted from N rates for each crop. Nitrogen fertilizer was applied to alfalfa from monoammonium phosphate at 11 kg N ha⁻¹ while applying as a P fertilizer. The N fertilization rate to durum was based on yield goal of 2350 kg ha⁻¹ and protein concentration of 135 g kg⁻¹. As per Montana State University recommendations, P fertilizer as monoammonium phosphate was applied to all crops at 56 kg P ha⁻¹ and K fertilizer as muriate of potash (60% K) at 48 kg K ha⁻¹ each year (Eastern Agricultural Research Center, 1997). All fertilizers were broadcast in 2002 and 2003 and banded to a depth of 5 cm below and to the side of seed rows in 2004 and 2005. No fertilizers were applied to the fallow phase in D-F. After fertilization, plots were tilled with a tandem disc in 2002 and a field cultivator equipped with C-shanks and 45-cm wide sweeps with coil-toothed harrows containing 60-cm bars to a depth of 8 cm from 2003 to 2005, except in alfalfa plots. In alfalfa plots, plots were tilled with tandem disc to a depth of 8 cm in 2002 and left

undisturbed thereafter from 2003 to 2005 when alfalfa was allowed to grow.

Immediately after fertilization and tillage, alfalfa (cultivar Shaw, Browning Brothers Seed, Mosby, MT) was planted at 9 kg ha⁻¹, durum (cultivar Mountrail, North Dakota State Univ. Foundation Seed, Fargo, ND) at 3 millions seeds ha⁻¹, barley hay (cultivar Haybet, Montana State Univ. Stock, Bozeman, MT) at 2.3 millions seeds ha⁻¹ in D-B and 1.6 million seeds ha⁻¹ in D-P/B, Austrian winter pea (cultivar Common, Circle S Seed, Logan, UT) at 0.8 millions seeds ha⁻¹, and foxtail millet hay (cultivar Golden German, Local Source, Williston, ND) at 28 kg ha⁻¹. In 2002 and 2003, all crops, except alfalfa, were planted with a drill equipped with double-disk opener at 19 cm row spacing. In 2004 and 2005, planting was done with a 3.1-m wide custom-built drill equipped with double-shoot Barton openers at 20 cm row spacing. Seeds were planted at depths of 1.9–7 cm, depending on crop species and soil water content at planting. Alfalfa was planted with a JD 750 drill at a row spacing of 20 cm in 2002. Growing season weeds in durum were controlled with labeled selective post-emergence herbicides. Contact herbicides were applied at postharvest as needed. Weeds in fallow plots were controlled by a combination of herbicides and tillage using sweeps three to four times in a year. No irrigation was applied. In July and August, 2002–2005, biomass (stems + leaves) yields of annual forages and durum were determined from two 0.5 m² areas randomly outside yield rows a day prior to grain harvest. Similarly, biomass yield of alfalfa was determined twice a year (July and October, 2002 to 2005) from two 0.5 m² areas. Total biomass yield of alfalfa in a year was determined by combining the two measurements. Yields were determined as dry matter weights following oven-drying at 55 °C for 3 d. Samples were ground to 1 mm for determinations of C and N concentrations. Durum grain yield was determined from an area of 2 m by 36 m on an oven-dried basis at 55 °C. After grain removal from the rest of the plots, biomass residue of durum was returned to the soil using a straw chopper and spreader. Biomass of forages was harvested for hay with a self-propelled smother and round baler.

2.3. Surface residue, root, and soil sample collection and analysis

After crop harvest in October, 2002–2005, soil samples containing roots were collected from the 0–120 cm depth from each plot using a hydraulic probe (5 cm inside diameter). Samples were collected from four places within each plot: one above the root crown, one between crowns in the crop row, and other two between rows. These were separated into 0–15, 15–30, 30–60, 60–90, and 90–120 cm increments to represent each depth and stored at 4 °C until roots were separated from the soil. In 2005, one additional undisturbed soil core was taken from the 0–120 cm depth from each plot, divided into segments to represent various depths as above, and bulk density was determined by dividing the weight of oven-dried soil at 110 °C by the volume of the core. Because of the difficulty of collecting undisturbed soil cores during dry season in the fall, only one of five cores containing relatively undisturbed soil was used for determining bulk density. Most cores contained disturbed soil samples and could not be used for determining bulk density. At the same time, soil surface crop residue samples were collected from all treatments from four 30 cm by 30 cm areas randomly in the central rows of the plot, composited, washed with water to remove soil, and dried in the oven at 60 °C for 3 d to obtain dry matter weight. Samples were ground to pass a 1 mm screen prior to C analysis.

Soil samples for all years, except for 2005, were thoroughly washed with water in a hydropneumatic elutriator containing 0.5-mm screen for several hours until all silt and clay particles were

washed out (Smucker et al., 1982). Roots and sand particles left in the screen were transferred into a container and coarse and fine live roots were hand-picked using a forceps. Because of the small amount of roots present at lower depths, roots from four cores within a treatment from the 0–120 cm depth were mixed, oven-dried at 55 °C for 3 d, weighed, and ground to 1 mm for C analysis. Root biomass at the 0–120 cm depth was calculated by dividing the weight of oven-dried roots by the area of soil core. For determining soil C fractions in 2005, about 20 g of soil subsample was collected from each core by weighing the soil, separating visible roots from the soil using a forceps, and returning roots back to the core samples. Visible root-free soil subsamples from four cores were composited within a depth, air-dried, and ground to 2 mm. Other soil samples were washed with water to determine root biomass yield and C concentration as above.

Carbon and N concentrations (g C or N kg⁻¹ plant dry weight) in above- (stems + leaves) and belowground (roots) biomass and soil surface residue were determined by dry combustion method using a high induction furnace C and N analyzer (LECO, St. Joseph, MI). The SOC in soil samples was determined by using the C and N analyzer as above after grinding the samples to <0.5 mm and pretreating the soil with 5% H₂SO₃ to remove inorganic C (Nelson and Sommers, 1996).

For determining PTC, 10 g soil sample was dispersed with 30 mL of 5 g L⁻¹ sodium hexametaphosphate by shaking for 16 h and the solution was poured through a 0.053 mm sieve (Cambardella and Elliott, 1992). The solution and particles that passed through the sieve and contained mineral associated and water soluble C were dried at 50 °C for 3 to 4 d and total C concentration was determined by using the analyzer as above. The PTC concentration was determined by the difference between total C in whole-soil and that in the particles that passed through the sieve after correcting for the sand content (Cambardella and Elliott, 1992). The PCM in air-dried soils was determined by the method modified by Haney et al. (2004). Ten grams of soil sample was moistened with water at 50% field capacity [0.25 m³ m⁻³ (Pikul and Aase, 2003)] and placed in a 1 L jar containing beakers with 2 mL of 0.5 M NaOH to trap evolved CO₂ and 20 mL of water to maintain high humidity. Soils were incubated in the jar at 21 °C for 10 d. At 10 d, the beaker containing NaOH was removed from the jar and PCM concentration was determined by measuring CO₂ absorbed in NaOH, which was back-titrated with 1.5 M BaCl₂ and 0.1 M HCl. The moist soil used for determining PCM was subsequently used for determining MBC by the modified fumigation-incubation method for air-dried soils (Franzluebbers et al., 1996). This container was incubated twice because MBC determination required moist-soil. The method also required mineralizable C to be flushed out during the first incubation (Franzluebbers et al., 1996). The moist soil was fumigated with ethanol-free chloroform for 24 h and placed in a 1 L jar containing beakers with 2 mL of 0.5 M NaOH and 20 mL water. As with PCM, fumigated moist soil was incubated for 10 d and CO₂ absorbed in NaOH was back-titrated with BaCl₂ and HCl. The MBC concentration was calculated by dividing the amount of CO₂-C absorbed in NaOH by a factor of 0.41 (Voroney and Paul, 1984) without subtracting the values from the nonfumigated control (Franzluebbers et al., 1996).

The contents (Mg C ha⁻¹ or kg C ha⁻¹) of SOC, PTC, PCM, and MBC at various depths were calculated by multiplying their concentrations (g C kg⁻¹ or mg C kg⁻¹) by bulk density and thickness of the soil layer. Since bulk density was not significantly influenced by treatments (data not shown), bulk density values of 1.45, 1.47, 1.44, 1.50, and 1.51 Mg m⁻³ at 0–15, 15–30, 30–60, 60–90, and 90–120 cm, respectively, averaged across treatments, were used to convert concentrations of soil C fractions into contents. The total contents at 0–120 cm were determined by summing the contents from individual depths.

2.4. Data analysis

Data for above- and belowground biomass yields and C contents, surface residue amount and C content, and soil C fractions were analyzed using the MIXED procedure of SAS after testing for homogeneity of variance (Littell et al., 1996). For above- and belowground biomass and C contents, cropping sequence was considered as the fixed effect, year as the repeated measure variable, and replication as the random effect. For surface residue C and soil C fractions at a depth, cropping sequence was considered as the fixed effect and replication as the random effect. Since each phase of the cropping sequence was present in every year, data for phases were averaged and used for a cropping sequence for analysis. Crops were absent during the fallow phase in D-F, therefore biomass yields and C contents for durum in this sequence were divided by 2 to determine the annualized values. Means were separated by using the least square means test when treatments and their interactions were significant (Littell et al., 1996). Statistical significance was evaluated at $P \leq 0.05$, unless otherwise stated.

3. Results and discussion

3.1. Crop biomass yield and carbon content

Since forages were harvested for hay, aboveground biomass and C returned to the soil in durum-annual forage sequences were contributed mainly by durum biomass (stems + leaves) after grain harvest, considering that leaf losses due to litterfall and harvest were negligible in annual forages. In alfalfa, the amount of leaves returned to the soil due to litterfall and harvest loss constitutes about 35% of the total aboveground biomass (Tomm et al., 1995). Therefore, aboveground biomass and C content in CA were contributed by litterfall and harvest loss of alfalfa.

Above- and belowground biomass yields and C contents varied significantly among cropping sequences and years, with a significant cropping sequence \times year interaction (data not shown). Aboveground biomass and C content were lower in CA than in other cropping sequences in all years, except in 2003 (Table 2). In durum-annual forage sequences, aboveground biomass was greater in D-F than in other sequences in 2003 and 2004. Similarly, biomass C was greater in D-F than in other sequences in 2004. In contrast, belowground biomass yield and C content were lower in D-F than in D-B, D-M, and D-P/B in 2002 and lower than in other sequences from 2003 to 2005. Compared with D-B, D-M, and D-P/B, belowground biomass and C content were lower in CA in 2002 but similar to or greater from 2003 to 2005. Averaged across cropping sequences, both above- and belowground biomass and C content were greater in 2004 and 2005 than in 2002.

While the lower aboveground biomass and C content in CA was due to removal of hay other than litterfall and harvest loss (Tomm et al., 1995), greater biomass and C content in D-F than in other cropping sequences was probably a result of greater water conservation and N availability due to increased N mineralization during fallow that increased succeeding crop grain and biomass yields (Aase and Pikul, 1995; Jones and Popham, 1997; Sainju et al., 2009). In contrast, lower belowground biomass and C content in D-F than in other cropping sequences was probably due to absence of crops during fallow. Slow growth of alfalfa reduced above- and belowground biomass and C content in 2002 but continuous root growth thereafter probably increased belowground biomass and C content in CA whose levels were similar to or greater than in other cropping sequences from 2003 to 2005. Root growth of annual forages and durum slows during physiological maturity (Merrill et al., 1996; Moroke et al., 2005), which, combined with their

Table 2

Effect of cropping sequence on annualized above- (leaves + stems) and belowground (root) crop biomass yields and C content returned to the soil from 2002 to 2005.

Cropping sequence ^a	2002	2003	2004	2005
Aboveground biomass yield (Mg ha ⁻¹) ^b				
CA	0.41b ^c	2.21b	1.38c	2.84b
D-B	2.25a	2.52b	3.31b	4.37a
D-F	2.68a	3.00a	5.00a	5.34a
D-M	2.46a	2.48b	2.92b	4.34a
D-P/B	2.65a	2.59b	3.46b	4.71a
Aboveground biomass C (Mg C ha ⁻¹) ^b				
CA	0.16b	0.91a	0.58c	1.14b
D-B	0.90a	1.01a	1.33b	1.78a
D-F	1.07a	1.19a	2.00a	2.14a
D-M	0.99a	0.99a	1.17b	1.73a
D-P/B	1.06a	1.04a	1.39b	1.84a
Belowground biomass yield (Mg ha ⁻¹) ^d				
CA	0.13c	0.68a	0.45a	0.90a
D-B	0.28b	0.32c	0.40a	0.56b
D-F	0.15c	0.17d	0.27b	0.29c
D-M	0.42a	0.45b	0.51a	0.78a
D-P/B	0.45a	0.40bc	0.55a	0.77a
Belowground biomass C (Mg C ha ⁻¹) ^d				
CA	0.05b	0.25a	0.17ab	0.35a
D-B	0.13a	0.13bc	0.15ab	0.20b
D-F	0.07b	0.06c	0.11b	0.11c
D-M	0.16a	0.17b	0.22a	0.30a
D-P/B	0.18a	0.15b	0.20ab	0.31a

^a Cropping sequences are CA, continuous alfalfa; D-B, durum-barley hay; D-F, durum-fallow; D-M, durum-foxtail millet hay; and D-P/B, durum-Austrian winter pea/barley mixture hay.

^b Aboveground biomass yield and C content included 35% of total biomass that was returned to the soil due to litterfall and harvest loss in CA (Tomm et al., 1995) and durum biomass in other cropping sequences.

^c Numbers followed by different letters within a column in a set are significantly different at $P \leq 0.05$ by the least square means test.

^d Belowground biomass yield and C content included both crown root and root biomass to a depth of 120 cm.

relatively short longevity, could have resulted in lower belowground biomass and C content in annual crops than in alfalfa.

The greater above- and belowground biomass yields and C contents in 2004 and 2005 than in 2002 was probably due to difference in the amount and distribution of precipitation. Total growing season (April to October) and annual precipitation were greater in 2004 and 2005 than in 2002 and the 105-yr average (Table 1). Precipitation during the growing season was also more uniformly distributed in 2004 and 2005 than in 2002. Growing season precipitation amount and distribution can influence dryland crop yields (Halvorson et al., 2002a; Sainju et al., 2009).

From 2002 to 2005, total aboveground biomass C was greater but belowground biomass C was lower in D-F than in other cropping sequences (Table 3). In contrast, total aboveground biomass C was lower but belowground biomass C (root C + rhizodeposit C) was similar to or greater in CA than in other cropping sequences. Total above- and belowground biomass C was lower in CA than in other cropping sequences, a result of removal of alfalfa aboveground biomass for hay. Differences in the amount of above- and belowground C inputs among cropping sequences are expected to influence surface residue and soil C fractions, as discussed below.

3.2. Soil surface residue amount and carbon content

Soil surface residue amount and C content in 2005 were significantly influenced by cropping sequence. Surface residue and C content were greater in D-F than in other cropping sequences (Table 4). Out of the total aboveground biomass C returned to the soil from 2002 to 2005, the amount of surface residue C in 2005 ranged from 14.9% in D-B to 20.1% in CA.

Table 3

Effect of cropping sequence on annualized total above- and belowground crop biomass (stems+leaves) C returned to the soil from 2002 to 2005.

Cropping sequence ^a	Total aboveground biomass C ^b (Mg C ha ⁻¹)	Total belowground biomass C	Estimated belowground rhizodeposit C	Total C input (Mg C ha ⁻¹)
		(0- to 120-cm) ^c (Mg C ha ⁻¹)	(0- to 120-cm) ^d (Mg C ha ⁻¹)	
CA	2.79c ^e	0.82a	0.55	4.16b
D-B	5.02b	0.61b	0.41	6.04a
D-F	6.40a	0.35c	0.23	6.98a
D-M	4.88b	0.85a	0.57	6.30a
D-P/B	5.33b	0.84a	0.56	6.73a

^a Cropping sequences are CA, continuous alfalfa; D-B, durum-barley hay; D-F, durum-fallow; D-M, durum-foxtail millet hay; and D-P/B, durum-Austrian winter pea/barley mixture hay.

^b Aboveground biomass C included 35% of total biomass C that was returned to the soil due to litterfall and harvest loss in CA (Tomm et al., 1995) and durum biomass C in other cropping sequences.

^c Belowground biomass C included both crown root and root biomass C to a depth of 120 cm.

^d Rhizodeposit C is estimated as 67% of root biomass C (Buyanovsky and Wagner, 1997).

^e Numbers followed by different letters within a column are significantly different at $P \leq 0.05$ by the least square means test.

Table 4

Effect of cropping sequence on soil surface residue and C content in 2005.

Cropping sequence ^a	Soil surface residue amount (Mg ha ⁻¹)	Soil surface residue C (Mg C ha ⁻¹)
CA	1.32b ^b	0.56b
D-B	1.79b	0.75b
D-F	2.62a	1.11a
D-M	1.75b	0.73b
D-P/B	1.92b	0.80b

^a Cropping sequences are CA, continuous alfalfa; D-B, durum-barley hay; D-F, durum-fallow; D-M, durum-foxtail millet hay; and D-P/B, durum-Austrian winter pea/barley mixture hay.

^b Numbers followed by different letters within a column are significantly different at $P \leq 0.05$ by the least square means test.

The greater surface residue amount and C content in D-F than in other cropping sequences could be a result of increased aboveground biomass and C returned to the soil (Tables 2 and 3) and greater C/N ratio. The C/N ratio of aboveground biomass returned to soil, averaged across years, was 30.2 in D-F compared with 10.2–27.7 in other cropping sequences. Similarly, C/N ratio of surface residue in 2005 was 79.3 in D-F compared with 14.0–53.3 in other cropping sequences. Residues with higher C/N ratio decompose slowly than residues with lower ratio (Kuo et al., 1997). Therefore, greater surface residue amount and C content in D-F could be the results of both greater C input and slower mineralization of the residue. Since the surface residue was collected in October 2005, accumulation of large amount of fresh biomass residue during durum phase in D-F also could have increased surface residue in D-F than in other treatments. Presence

of surface residue in CA shows that the residue was probably contributed by litterfall and harvest loss of alfalfa (Tomm et al., 1995). Soil surface residue amount is linearly related with residue cover (Sainju et al., 2007), which in turn, can reduce the potential for soil erosion (Fryrear, 1985). Since 2 Mg ha⁻¹ of surface residue is needed to effectively control soil erosion (Fryrear, 1985), D-F and D-P/B may be able to reduce erosion better than other cropping sequences.

3.3. Soil organic carbon and particulate total carbon

The SOC and PTC contents in 2005 varied significantly with cropping sequences at multiple soil depths. At 0–15 cm, SOC was greater in CA than in D-B and D-M (Table 5). At other depths, SOC was not influenced by treatment. The PTC at 0–15 cm was greater in CA than in other cropping sequences. At 30–60 cm, PTC was greater in D-B than in CA, D-M, and D-P/B. At 60–90, 90–120, and 0–120 cm, PTC was greater in D-P/B than in D-B. The proportion of SOC in PTC (PTC/SOC ratio), i.e. the proportion of soil organic C in coarse fraction, at 0–120 cm was greater in D-P/B than in other cropping sequences (Table 6).

It has been reported that SOC levels normally do not change after 5–8 yr of tillage and cropping sequence under dryland cropping systems in the central and northern Great Plains, USA (Halvorson et al., 2002a,b; Ortega et al., 2002; Sainju et al., 2006a). This is because the amount of aboveground biomass residue returned to the soil is lower in dryland than in irrigated cropping systems (Halvorson et al., 2002b; Sainju et al., 2006a). Furthermore, limited precipitation and cold weather in the central and northern Great Plains slow the turnover rate of plant C into soil C

Table 5

Effect of cropping sequence on soil organic C (SOC) and particulate total C (PTC) contents at the 0–120 cm depth in 2005.

Cropping sequence ^a	Soil depth								
	0–15 cm	15–30 cm	30–60 cm	60–90 cm	90–120 cm	0–30 cm	0–60 cm	0–90 cm	0–120 cm
SOC content (Mg C ha ⁻¹)									
CA	18.7a ^b	13.3a	25.7a	49.3a	48.8a	32.0a	57.7a	107.0a	155.8a
D-B	13.8b	14.4a	40.0a	48.9a	49.3a	28.2a	68.2a	117.1a	166.4a
D-F	15.9ab	15.6a	35.7a	56.9a	53.9a	31.5a	67.2a	124.1a	178.0a
D-M	13.5b	14.7a	30.5a	55.1a	52.7a	28.2a	58.7a	113.8a	166.5a
D-P/B	16.5ab	13.8a	32.4a	44.9a	40.2a	30.3a	62.7a	107.6a	147.8a
PTC content (Mg C ha ⁻¹)									
CA	4.8a	3.2a	18.7b	25.8b	29.6ab	8.0a	26.7ab	52.5ab	82.1ab
D-B	3.7b	3.8a	22.4a	19.5c	23.5bc	7.5ab	29.9a	49.4b	72.9b
D-F	3.6b	3.0a	19.2ab	32.8a	19.5c	6.6b	25.8ab	58.6a	78.1ab
D-M	3.7b	3.7a	17.9b	27.1b	24.7b	7.4ab	25.3ab	52.4ab	77.1ab
D-P/B	3.7b	3.1a	14.3c	34.1a	33.8a	6.8ab	21.2b	55.2ab	89.1a

^a Cropping sequences are CA, continuous alfalfa; D-B, durum-barley hay; D-F, durum-fallow; D-M, durum-foxtail millet hay; and D-P/B, durum-Austrian winter pea/barley mixture hay.

^b Numbers followed by different letters within a column in a set are significantly different at $P \leq 0.05$ by the least square means test.

Table 6

Effect of cropping sequence on the proportion of soil organic C (SOC) in particulate total C (PTC), potential C mineralization (PCM), and microbial biomass C (MBC), and proportion of MBC in PCM at the 0–120 cm depth in 2005.

Cropping sequence ^a	PTC/SOC ratio (g PTC kg ⁻¹ SOC)	PCM/SOC ratio (g PCM kg ⁻¹ SOC)	MBC/SOC ratio (g MBC kg ⁻¹ SOC)	PCM/MBC ratio (g PCM kg ⁻¹ MBC)
CA	527b ^b	2.25a	5.24ab	429a
D-B	438c	1.61c	4.42b	364ab
D-F	439c	1.51c	4.47b	338b
D-M	463bc	1.80bc	4.61b	390ab
D-P/B	603a	2.06ab	5.48a	376ab

^a Cropping sequences are CA, continuous alfalfa; D-B, durum-barley hay; D-F, durum-fallow; D-M, durum-foxtail millet hay; and D-P/B, durum-Austrian winter pea/barley mixture hay.

^b Numbers followed by different letters within a column are significantly different at $P \leq 0.05$ by the least square means test.

(Halvorson et al., 2002a; Sainju et al., 2006a). The greater SOC at 0–15 cm in CA than in other cropping systems after 4 yr in this experiment, however, could be the results of greater belowground biomass C and a relatively undisturbed soil condition, although aboveground biomass C was lower (Table 3). It could be possible that alfalfa roots played a greater role in maintaining surface soil C levels than aboveground biomass in CA (Balesdent and Balabane, 1996; Puget and Drinkwater, 2001). Although plots in all treatments were tilled at planting in 2002, alfalfa was grown in a relatively undisturbed soil condition thereafter in CA. Several studies (Russell et al., 2005; Syswerda et al., 2011; VandenBygaart et al., 2011) have also reported that SOC was greater in alfalfa than in annual crops or greater in rotations containing alfalfa with cereals than in continuous cereal cropping. In contrast, plots in durum-annual forage sequences were tilled several times a year for planting and to control weeds from 2002 to 2005. Tillage can reduce soil organic C levels due to mineralization as a result of residue incorporation, aggregate disruption, and increased aeration (Schomberg and Jones, 1999; Halvorson et al., 2002b). The SOC levels below the 15 cm depth or in the whole soil profile (0–120 cm), however, were not altered by cropping sequence. Greater variations in SOC levels in subsoil layers than in the surface soil have been known to show nonsignificant effect of cropping sequence on SOC in the underlying layers or in the whole soil profile (Kravchenko and Robertson, 2011; Syswerda et al., 2011; VandenBygaart et al., 2011).

Since the original SOC level at 0–15 cm prior to the initiation of the experiment in 2001 was 17.4 Mg C ha⁻¹, SOC increased by 1.3 Mg C ha⁻¹ in CA but decreased by 0.9–3.9 Mg C ha⁻¹ in other treatments in 2005. This suggests that C can be sequestered at 325 kg C ha⁻¹ yr⁻¹ by planting annual legume forages, such as alfalfa, at the surface 15 cm soil layer under dryland farming systems in the northern Great Plains. Halvorson et al. (2002b)

reported that no-tilled continuous cropping increased dryland C sequestration by 233 kg C ha⁻¹ yr⁻¹ compared with a loss of 141 kg C ha⁻¹ yr⁻¹ in conventional-tilled crop-fallow system after 10 yr in the northern Great Plains. Conversion of annual cropping to perennial cropping systems can sequester as much as 600 kg C ha⁻¹ yr⁻¹ (Council for Agricultural Science and Technology, 2004; Syswerda et al., 2011; VandenBygaart et al., 2011).

Similar to SOC, greater PTC at 0–15 cm in CA than in other cropping sequences could be a result of greater belowground biomass C and a relatively undisturbed soil condition. Russell et al. (2005) and Bremer et al. (2007) reported that crop rotations containing alfalfa and perennial grasses increased PTC compared with annual crops. The PTC below the 30 cm depth varied more with cropping sequences than SOC whose reasons were not clear. It could be possible that greater variability in SOC levels in the subsoil horizons among treatments (Kravchenko and Robertson, 2011; Syswerda et al., 2011; VandenBygaart et al., 2011) could also have resulted in greater PTC variation below 30 cm. It has been known that PTC vary rapidly with cropping sequences than SOC over time (Cambardella and Elliott, 1992; Sainju et al., 2007). Greater proportion of SOC in PTC at 0–120 cm in D-P/B than in other cropping sequences indicates that most of SOC in this treatment occurs in coarse size fraction. The reasons for this were not known. The increased PTC/SOC ratio with soil depth, averaged across treatments (248 g PTC kg⁻¹ SOC at 0–15 cm to 534 g PTC kg⁻¹ SOC at 90–120 cm) was probably a result of increased inorganic C content with depth, since PTC contained both organic and inorganic C while SOC contained only organic C. Substantial amount of soil inorganic C has been known to occur below the 30 cm depth in the dryland cropping systems in the northern Great Plains (Cihacek and Ulmer, 2002; Monger, 2002; Sainju et al., 2007).

Table 7

Effect of cropping sequence on soil potential C mineralization (PCM) and microbial biomass C (MBC) contents at the 0–120 cm depth in 2005.

Cropping sequence ^a	Soil depth								
	0–15 cm	15–30 cm	30–60 cm	60–90 cm	90–120 cm	0–30 cm	0–60 cm	0–90 cm	0–120 cm
	PCM content (kg C ha ^{−1})								
CA	67.9a ^b	58.4a	93.0a	73.6a	58.1a	126.3a	219.3a	292.9a	351.0a
D-B	57.0b	47.5ab	66.0c	56.2bc	40.9b	104.5b	170.5bc	226.7b	267.6c
D-F	54.3b	41.7b	71.2c	60.3b	41.5b	96.0b	167.2c	227.5b	269.0c
D-M	55.6b	54.5a	83.7ab	46.1c	60.5a	110.1b	193.8b	239.7b	300.4b
D-P/B	53.5b	49.9ab	76.6bc	69.5ab	55.4a	103.4b	180.0bc	249.5ab	304.9b
	MBC content (kg C ha ^{−1})								
CA	114a	106a	195ab	211a	190a	220a	415a	626ab	816a
D-B	133a	120a	186b	152c	145b	253a	439a	591b	736b
D-F	119a	115a	203ab	197ab	162ab	234a	437a	634ab	796ab
D-M	116a	119a	195ab	176bc	162ab	235a	430a	606b	768ab
D-P/B	126a	104a	215a	197ab	168ab	230a	445a	642a	810ab

^a Cropping sequences are CA, continuous alfalfa; D-B, durum-barley hay; D-F, durum-fallow; D-M, durum-foxtail millet hay; and D-P/B, durum-Austrian winter pea/barley mixture hay.

^b Numbers followed by different letters within a column in a set are significantly different at $P \leq 0.05$ by the least square means test.

3.4. Soil potential carbon mineralization and microbial biomass carbon

Similar to other C fractions, PCM and MBC contents in 2005 varied with cropping sequences at various soil depths. The PCM at 0–15, 0–30, 0–60, 0–90, and 0–120 cm was greater in CA than in other cropping sequences (Table 7). At 15–30 and 90–120 cm, PCM was greater in CA and D-M than in D-F. At 30–60 and 60–90 cm, PCM was greater in CA than in D-B and D-F. At 0–60 and 0–120 cm, PCM was also greater in D-M than in D-F. The proportion of SOC in PCM (PCM/SOC ratio), i.e. the proportion of soil organic C in the mineralizable form, at 0–120 cm was greater in CA than in D-B, D-F, and D-M and greater in D-P/B than in D-B and D-F (Table 6).

The MBC at 30–60 cm was greater in D-P/B than in D-B, but at 60–90 and 90–120 cm was greater in CA than in D-B (Table 7). At 0–90 cm, MBC was greater in D-P/B than in D-B and D-M, but at 0–120 cm was greater in CA than in D-B. The proportion of SOC in MBC (MBC/SOC ratio), i.e. the proportion of soil organic C in microbial biomass, at 0–120 cm was greater in D-P/B than in D-B, D-F, and D-M (Table 6). The proportion of MBC in PCM (PCM/MBC ratio), i.e. the proportion microbial biomass C in the mineralizable form, at 0–120 cm was greater in CA than in D-F.

The greater PCM and MBC in CA than in other cropping sequences indicate that perennial legume forages, such as alfalfa, increased soil microbial biomass and activity compared with annual crops, probably due to increased root biomass and rhizodeposit C input (Table 3), followed by a relatively undisturbed soil condition and continuous root growth throughout the year, a case similar to that observed for SOC and POC. While increase in PCM with CA was observed throughout the soil profile (0–120 cm), increase in MBC occurred only at the subsoil layers (60–120 cm). It may be possible that, similar to SOC and POC, root and rhizodeposit C may have played greater roles in stimulating microbial biomass and activity in the subsoil layers than aboveground biomass C (Balesdent and Balabane, 1996; Puget and Drinkwater, 2001). Substantial increase in PCM at the surface layer (0–15 cm) with CA was probably a result of increased substrate availability, since SOC and POC were also greater with this treatment at this layer. Sainju et al. (2003, 2006b) found that perennial legume forages, such as rhizoma peanut (*Arachis glabrata* Benth.), increased PCM and MBC at 0–90 cm compared with perennial weeds due to greater root growth. They concluded that even with the harvest of aboveground biomass for hay, rhizoma peanut improved soil quality and productivity due to increased microbial biomass and activity compared with perennial weeds where aboveground biomass was returned to the soil.

The lower PCM and MBC in D-B and D-F than in other cropping sequences may be related to lower belowground biomass and rhizodeposit C input (Table 3), followed by their higher above- and belowground biomass C/N ratio (26.6 and 30.2 for aboveground biomass and 35.8 and 58.5 for belowground biomass in D-B and D-F, respectively, compared with 10.2–27.7 and 21.0–49.4 for other treatments). While microbial biomass and activity are related to the amount of substrate availability (Franzluebbers et al., 1995; Bezdicsek et al., 1996; Sainju et al., 2007), residues with higher C/N ratio decompose slowly (Kuo et al., 1997) and therefore may reduce microbial activity. The lower PCM/SOC and MBC/SOC ratios in D-B and D-F than in other treatments (Table 6) shows that proportion of soil organic C in the microbial biomass and mineralizable form are lower in these treatments. In contrast, greater PCM/SOC and MBC/SOC ratios in CA and D-P/B were probably related to lower C/N ratio of root biomass (21.0 and 27.4, respectively) that may have promoted microbial biomass and activity in these treatments. The greater PCM/MBC ratio in CA also shows that most of the microbial biomass in this treatment is mineralizable. Similarly, greater levels of PTC/SOC and MBC/SOC

ratios in D-P/B than in other treatments or similar order of sequence among treatments (Table 6) indicates that PTC may act as substrate to microbial biomass (Six et al., 1999).

Compared with the surface soil layer, PCM and MBC below the 30 cm depth seemed to be relatively higher. Although greater soil bulk density with depth and thickness of soil layer increased PCM and MBC contents at the subsurface layers, higher soil inorganic C, as shown by increased PTC with depth (Table 5), also may have resulted in greater PCM and MBC in these layers. Further studies are needed to verify if increased soil inorganic C content with depth will also increase PCM and MBC.

4. Conclusions

Forages grown in rotation with or without durum influenced above- and belowground biomass C inputs and soil C fractions. Alfalfa, grown alone, increased belowground but reduced aboveground biomass C input compared with durum-annual forage sequences due to harvest of aboveground biomass for hay. In contrast, durum-annual forage sequences increased aboveground biomass C due to durum biomass returned to the soil after grain harvest. As a result, SOC and PTC at the 0–15 cm depth and PCM and MBC at 0–120 cm were greater under alfalfa than under durum-annual forage sequences. Perennial legume forages, such as alfalfa, may increase soil C storage at $325 \text{ kg C ha}^{-1} \text{ yr}^{-1}$ at the surface layer and microbial biomass and activities at surface and subsurface layers due to greater belowground biomass C input and a relatively undisturbed soil condition compared with durum-annual forage sequences where plots were tilled several times a year for planting and weed control. Even though aboveground biomass of alfalfa was removed for hay, roots may play a greater role in soil C dynamics and storage than aboveground biomass. Because of continuous root growth throughout the year and greater root and rhizosphere root C input, alfalfa may increase C sequestration and soil quality due to increased microbial biomass and activity compared with durum-annual forage sequences. Greater aboveground biomass C, however, increased C storage in surface residue but reduced soil microbial biomass and activity under durum-fallow than under durum-annual forage sequences.

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